

What we claim is:

1. A prepared DNA segment for placement in a suitable expression vector and transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate proteoglycan entities subsequently occurs in-situ, said prepared DNA segment comprising:

at least one first DNA sequence coding for the extracellular domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of an expressed proteoglycan entity which is then located at and extends from the endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ;

at least one second DNA sequence coding for the transmembrane domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said transmembrane domain second DNA sequence specifying the medial portion of an expressed proteoglycan entity which is then located at and extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed proteoglycan entity; and

at least one third DNA sequence coding for the cytoplasmic domain of the syndecan-4 molecule in said discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of an expressed proteoglycan entity which is then present within the cytoplasm of a transfected endothelial cell and is

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joined to said transmembrane portion and said extracellular N-terminal portion of said expressed proteoglycan entity.

2. A constructed expression vector for transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate proteoglycan entities subsequently occurs in-situ, said constructed expression vector comprising:

a prepared DNA segment comprised of

(i) at least one first DNA sequence coding for the extracellular domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of an expressed proteoglycan entity which is then located at and extends from the endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ,

(ii) at least one second DNA sequence coding for the transmembrane domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said transmembrane domain second DNA sequence specifying the medial portion of an expressed proteoglycan entity which is then located at and extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed proteoglycan entity, and

(iii) at least one third DNA sequence coding for the cytoplasmic domain of the syndecan-4 molecule in said discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said syndecan-4 cytoplasmic

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domain third DNA sequence specifying the cytoplasmic portion of an expressed proteoglycan entity which is then present within the cytoplasm of a transfected endothelial cell and is joined to said transmembrane portion and said extracellular N-terminal portion of said expressed proteoglycan entity; and

an expression vector carrying said prepared DNA segment and suitable for transfection of endothelial cells in-situ.

3. An in-situ transfected endothelial cell which overexpresses extracellular matrix heparan sulfate proteoglycans and positions on the proteoglycan entities at the cell surface, said in-situ transfected endothelial cell comprising:

✓ a viable endothelial cell previously transfected in-situ with a constructed expression vector such that said transfected endothelial cell overexpresses discrete extracellular matrix heparan sulfate proteoglycan entities coded for by said vector, said overexpressed proteoglycan entities being comprised of

(i) an extracellular N-terminal portion which is located at and extends from the transfected endothelial cell surface and which binds heparan sulfates to form an extracellular matrix in-situ, said extracellular N-terminal portion being the expressed product of at least one first DNA sequence in the prepared expression vector coding for the extracellular domain of said proteoglycan entity expressed by the transfected endothelial cell in-situ,

(ii) a transmembrane medial portion which is located at and extends through the endothelial cell membrane and is joined with said extracellular N-terminal portion of said proteoglycan entity, said transmembrane medial portion

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being the expressed product of at least one second DNA sequence in the prepared expression vector coding for the transmembrane domain of said proteoglycan entity expressed by the transfected endothelial cell in-situ, and

(iii) a syndecan-4 cytoplasmic portion present within the cytoplasm of the transfected endothelial cell which is joined to said transmembrane portion and said extracellular N-terminal portion of said proteoglycan entity, said syndecan-4 cytoplasmic portion being the expressed product of at least one third DNA sequence in the prepared expression vector coding for the cytoplasmic domain of the syndecan-4 molecule of said proteoglycan entity expressed by the transfected endothelial cell in-situ.

4. The prepared DNA segment as recited by claim 1 wherein said first DNA sequence coding for the extracellular domain of a discrete proteoglycan entity is selected from the group consisting of syndecan DNA sequences, glypican DNA sequences and perlecan DNA sequences.

5. The prepared DNA segment as recited by claim 1 wherein said second DNA sequence coding for the transmembrane domain of a discrete proteoglycan entity is selected from the group consisting of syndecan DNA sequences, glypican DNA sequences and perlecan DNA sequences.

6. The constructed expression vector as recited by claim 2 wherein said expression vector suitable for transfection of endothelial cells in-situ is a plasmid.

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7. The constructed expression vector as recited by claim 2 wherein said expression vector suitable for transfection of endothelial cells in-situ is a virus.

8. The in-situ transfected endothelial cell as recited by claim 3 wherein said cell is selected from the group consisting of vascular endothelial cells and dermal endothelial cells.

9. The in-situ transfected endothelial cell as recited by claim 3 wherein said cell exists under in-vivo conditions.

10. The in-situ transfected endothelial cell as recited by claim 3 wherein said cell exists under in-vitro conditions.

11. The in-situ transfected endothelial cell as recited by claim 3 wherein said transfected endothelial cell exists in a tissue comprising at least one kind of muscle cell selected from the group consisting of myocardial muscle cells, smooth muscle cells and striated muscle cells.

12. A method for making a prepared DNA segment intended for placement in a suitable expression vector and transfection of endothelial cells in-situ such that overexpression of extracellular matrix heparan sulfate proteoglycan entities subsequently occurs in-situ, said method comprising the steps of:

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joining together said extracellular domain first DNA sequence, said transmembrane domain second DNA sequence, and said syndecan-4 cytoplasmic domain third DNA sequence as a discrete prepared DNA segment.

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13. A method for making a constructed expression vector intended for transfection of endothelial cells in-situ such that overexpression of extracellular matrix haparan sulfate proteoglycans subsequently occurs in-situ, said method comprising the step of:

obtaining a prepared DNA segment comprised of

(i) at least one first DNA sequence coding for the extracellular domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said extracellular domain first DNA sequence specifying the extracellular N-terminal portion of an expressed proteoglycan entity which is then located at and extends from the transfected endothelial cell surface and is capable of binding heparan sulfates to form an extracellular matrix in-situ,

(ii) at least one second DNA sequence coding for the transmembrane domain of a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said transmembrane domain second DNA sequence specifying the medial portion of an expressed proteoglycan entity which is then located at and extends through the transfected endothelial cell membrane and is joined with said extracellular N-terminal portion of said expressed proteoglycan entity, and

(iii) at least one third DNA sequence coding for the cytoplasmic domain of the syndecan-4 molecule in a discrete proteoglycan entity that is expressed by a transfected endothelial cell in-situ, said syndecan-4 cytoplasmic domain third DNA sequence specifying the cytoplasmic portion of an expressed proteoglycan entity which is then present within the cytoplasm of a transfected

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endothelial cell and is joined to said transmembrane portion and said extracellular N-terminal portion of said expressed proteoglycan entity; and

positioning said prepared DNA segment in an expression vector suitable for transfection of endothelial cells in-situ.

14. A method for stimulating angiogenesis in-situ within a living tissue comprising vascular endothelial cells, said method comprising the steps of:

transfecting vascular endothelial cells within a living tissue with a constructed expression vector such that the resulting transfected endothelial vascular cells overexpress discrete extracellular matrix heparan sulfate proteoglycan entities coded for by said constructed expression vector, said overexpressed proteoglycan entities being comprised of

- (i) an extracellular N-terminal portion which is located at and extends from the transfected vascular endothelial cell surface and binds heparan sulfates to form an extracellular matrix in-situ, said extracellular N-terminal portion being the expressed product of at least one first DNA sequence in the constructed expression vector coding for the extracellular domain of said proteoglycan entity expressed by a transfected endothelial cell in-situ,
- (ii) a transmembrane medial portion which is located at and extends through a transfected vascular endothelial cell membrane and is joined with said extracellular N-terminal portion of expressed proteoglycan entity, said transmembrane medial portion being the expressed product of at least one second DNA sequence in the constructed expression vector coding for the transmembrane

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domain of said proteoglycan entity expressed by a transfected endothelial cell in-situ, and

(iii) a syndecan-4 cytoplasmic portion present within the cytoplasm of a transfected endothelial cell which is joined to said transmembrane portion and said extracellular N-terminal portion of said proteoglycan entity, said syndecan-4 cytoplasmic portion being the expressed product of at least one third DNA sequence in the constructed expression vector coding for the cytoplasmic domain of the syndecan-4 molecule of said proteoglycan entity expressed by a transfected endothelial cell in-situ; and

allowing said transfected vascular endothelial cells bearing said overexpressed extracellular matrix proteoglycan entities to stimulate angiogenesis in-situ.

15. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprises at least one other type of cell selected from the group consisting of muscle cells, fibrocytes and fibroblasts, epithelial cells, osteocysts and osteoblasts, erythrocytes and leukocytes, and neurons.

16. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprises at least one tissue selected from the group consisting of myocardium, lung, brain, kidney, spleen, liver, and gastro-intestinal tissues.

17. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said living tissue comprising vascular endothelial cells is transfected using means selected from the group consisting of catheter-based administration, injection-based administration, infusion-based administration, localized intravascular deliveries, liposome-based deliveries, and administrations using target-directed peptides.

18. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said method is practiced under in-vivo conditions.

19. The method for stimulating angiogenesis in-situ as recited by claim 14 wherein said method is practiced under in-vitro conditions.

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